**Remarks/Arguments:**

## I. Status of the Application

Claim 47 is amended herein. Support for the claim amendment is found throughout the application as originally filed, including, for example, paragraphs [0016], [0017], [0027]-[0030] and Example 1. Claim 55 has also been amended to be consistent with the amendment to claim 47. No new matter has been introduced. Claims 13-19, 21-27, 35-37 and 44-46 are withdrawn from consideration as a result of a restriction requirement. Claims 1-12, 20, 28-34, 38-43 and 53 have been cancelled. Consequently, claims 47-52 and 54-60 remain pending and under examination in the application.

## II. Rejections under 35 U.S.C. § 103

II.1.- Rejection over *Kumazawa et al.*, in view of *Ragland*

Claims 47-49 and 51-55 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious over *Kumazawa et al.* (Japan J Microbiol 1976; 20:183-190) in view of *Ragland* (US 4,744,984).

Claim 47 is the only independent claim currently pending and under examination in the present application. Claim 47 is directed to an immunotherapeutic agent comprising cell wall fragments from a virulent *Mycobacterium tuberculosis*-complex (MTB-C) strain of cells obtained by a process consisting essentially of the steps of:

a) culturing the cells for a period of at least three weeks,

b) homogenizing the cells in the presence of a non-ionic surfactant to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds, wherein the non-ionic surfactant is selected from the group consisting of alkylphenol ethoxylates and ethoxylated sorbitan esters,

c) centrifuging the homogenate to separate the cell wall fragments from the non-fragmented cells and the solubilized cell compounds,

d) washing the cell wall fragments and further inactivating any remaining virulent cells to obtain the immunotherapeutic agent, and

e) optionally, lyophilizing the immunotherapeutic agent;

wherein the immunotherapeutic agent is substantially free of non-fragmented cells and solubilized cell compounds.

The Applicant respectfully submits that the amendment of claim 47 overcomes the obviousness rejection for the following reasons.

*i) Kumazawa discloses the preparation of a water-soluble extract obtained by chemical hydrogenolysis of bacterial cells, but not the preparation of cell wall fragments*

In contrast, claim 47 requires that the immunotherapeutic agent of the present patent application is constituted by insoluble cell wall fragments, but not non-fragmented cells or solubilized cell compounds, because:

1) according to step c) of the recited process, the cell wall fragments present in the immunotherapeutic agent have been separated from the non-fragmented cells and the solubilized cell compounds, and
2) there is a further recitation in claim 47 that the immunotherapeutic agent of the present patent application is free of non-fragmented cells and solubilized cell compounds.

*ii) Ragland discloses the preparation of an antiviral immunotherapeutic agent comprising deproteinized cell walls and free of lipids and nucleic acids*

*Ragland* is directed to the preparation of an antiviral immunotherapeutic agent, which comprises an effective amount of a deproteinized mycobacterial cell wall suspension, from which nucleic acids and lipids have been extracted by treatment with urea, detergent and phenol.

There are several significant differences between the process recited in amended claim 47 and the process disclosed by *Ragland*:

1) In Applicant's invention, the cells are cultured for a period of at least three weeks, and not between 10 to 20 days as taught by *Ragland*.

2) The process of the present patent application does not include a step of deproteinizing the cell wall fragments by the treatment with proteinases as in the process disclosed by *Ragland*.

3) The process of the present patent application does not include a step of removing lipids and nucleic acids by treatment with urea, detergent and phenol as in the process disclosed by *Ragland*.

These processing differences lead to structural and antigenic differences between the immunotherapeutic agent of the present patent application and the active agent disclosed by *Ragland*:

1) The final active immunotherapeutic agent of the present patent application comprises insoluble cell wall fragments, proteins, lipids and nucleic acids, whereas the final active drug obtained by the process disclosed by *Ragland* comprises deproteinized cell wall fragments without proteins, nucleic acids and lipids (see *Ragland*, Example 3, Example 4, claim 3).

2) The immunogenic response of the immunotherapeutic agent of the present patent application is directed to a <u>bacterial</u> infection, i.e. tuberculosis, whereas the active agent disclosed by *Ragland* shows an unspecific immunogenic response directed to any <u>viral</u> infection (see *Ragland*, column 3, lines 32 to 38).

3) The immunogenic response of the immunotherapeutic agent of the present patent application is directed to a bacterial infection, i.e. tuberculosis, whereas the active agent disclosed by *Ragland* does not sensitize the host to tuberculin skin tests (see *Ragland*, column 3, lines 53 to 55).

These structural and antigenic differences result from the specific process recited in amended claim 47 as explained below.

The recited culture time of at least 3 weeks, preferably from 3 to 4 weeks, is a feature which results in structural differences. The relatively long claimed culturing time leads to the induction of microaerobic (or stressful) conditions similar to those that are thought to be found in granulomas that contain *M. tuberculosis* latent bacilli and that therefore may favor the production of antigens typical from these latent bacilli. In fact, when increasing the culturing time to at least 3 weeks, which is not optimal for achieving maximal cell density and viability, a different antigenic protein profile is observed. These results are not obtained with a lower culturing time.

The deproteinization and the removal of nucleic acids and lipids from the cell wall fragments in the process disclosed by *Ragland* lead to substantially different cell wall fragments from those obtained by the process recited in amended claim 47. They will differ mainly in the lipidic and protein content as well as in the way the remaining lipids and proteins are presented in comparison with cell wall fragments, which were further deproteinized and treated with urea, detergent and phenol to remove nucleic acids and lipids. Such differences in the composition result also in different biological activities: the immunotherapeutic agent of the present patent application is active specifically against tuberculosis, a bacterial infection, whereas the active agent obtained according to the process disclosed by *Ragland* is unspecifically active against viral infections.

As claims 48, 49, 51, 52, 54 and 55 each depend, directly or indirectly, from claim 47 and thus incorporate all the limitations of the base claim, they, too, are patentably unobvious over the cited references for at least the same reasons as claim 47. Reconsideration and withdrawal of the rejection are respectfully requested.

II.2.- Rejection over *Kumazawa et al.* in view of *Ragland* and further in view of *Mohr et al.*

Claim 50 stands rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over *Kumazawa et al.* in view of *Ragland*, and further in view of *Mohr et al.* (US 7,214,651).

Claim 50 is dependent from claim 49, which in turn is dependent from claim 47. Since, as discussed above, neither claim 49 or claim 47 is obvious over *Kumazawa* in view of *Ragland*, then dependent claim 50, which further limits the immunotherapeutic agent, must also be non-obvious over those references. The Mohr et al. reference fails to cure the deficiencies of Kumazawa and Ragland which were previously noted, because it does not teach or suggest any method for preparing cell wall fragments having the features of the method utilized in Applicant's invention, whereby an immunotherapeutic agent substantially free of non-fragmented cells and solubilized cell compounds is obtained.

For these reasons, the Applicant respectfully requests that the Examiner withdraw the rejection.

II.3.- Rejection over *Kumazawa et al.* in view of *Ragland* and further in view of *Lyons et al.*

Claims 47-49 and 51-55 were rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over *Kumazawa et al.* in view of *Ragland*, and further in view of *Lyons et al.* (Infect Immunity 2002; 70:5471-8).

According to the Examiner, *Lyons* supplements the combined teaching of *Kumazawa* and *Ragland* by establishing it is well known in the art that the cell wall extract on *Mycobacteria* strain H37Rv has been used as a vaccine composition for treating *M. tuberculosis* infection.

The Examiner concludes that it would have been obvious to the skilled person to use either *M. tuberculosis* strain Aoyama B as taught by *Kumazawa* or strain H37Rv as taught by *Lyons* with a reasonable expectation of success.

The Applicant respectfully disagrees.

The deficiencies of the Kumazawa and Ragland references with respect to sole independent claim 47 were addressed previously in Section II.2. These deficiencies are not remedied by the disclosure of *Lyons et al.*

*Lyons et al.* discloses investigations related to understanding how prior vaccination may alter very early events in the *M. tuberculosis*-infected lung. In this context, *Lyons et al.* demonstrate that primary alveolar macrophages harvested from BCG-vaccinated guinea pigs and stimulated

in vitro with a variety of stimuli including cell walls of virulent strain H37Rv produce significantly higher levels of mRNA and protein for IL-8 than do alveolar macrophages harvested from nonvaccinated guinea pigs.

*Lyons et al.* does not disclose any method for preparing cell walls because they were provided by the laboratory of John Belisle from the Department of Microbiology, Colorado State University, Fort Collins.

According to the information available on the Web page of that University regarding Tuberculosis Vaccine Testing and Research Materials Contract (this document was attached as Exhibit B to the previous Amendment submitted April 13, 2011), that cell wall fraction likely had been prepared in the following way:

> **Reagent:**
> Cell Wall Fraction, CW
> **Default Quantity:**
> 1 mg
> **Production system:**
> Each strain is grown to late-log phase (day 14) in glycerol-alanine-salts (GAS) medium, washed with PBS pH 7.4 and inactivated by gamma-irradiation. The bacilli are suspended (2 g/ml) in PBS containing 8 mM EDTA, DNase, RNase and a proteinase inhibitor tablet, and broken in a French Press pressure cell at 4°C. Unbroken cells are removed by low speed (3,000 x g) centrifugation. The cell wall is isolated by centrifugation at 27,000 x g for one hour and washed 2 times in PBS. The final cell wall pellet is suspended and dialyzed in 0.01M ammonium bicarbonate, quantified by BCA protein assay for protein content, and stored at -80°C.
> **Notes:**
> This preparation contains proteins and non-protein compounds such as mAGP.

This process used for preparing the cell wall fragments differs substantially from the process of the present patent application, as shown in the following table:

| Process steps | *Lyons et al.* | Patent application Example 1 |
|---|---|---|
| 1 | MTB-C were cultured for 14 days | MTB-C cells are cultured for at least 3 weeks |
| 2 | Cells were inactivated by gamma-irradiation | - |
| 3 | Cells were disrupted by pressure in the presence of DNase, RNase and proteinase inhibitor | Cells are homogenized in the presence of a non-ionic surfactant selected from the group consisting of alkylphenol ethoxylates and sorbitan ester ethoxylates |
| 3 | Insoluble cell wall fragments were separated from non-fragmented cells and solubilized cell compounds, and washed | Insoluble cell wall fragments were separated from non-fragmented cells and solubilized cell compounds, washed and inactivated |

From the above table, it is apparent that the process of the present patent application differs substantially from the process disclosed by reference in *Lyons et al*. This process differs in two essential points: culturing time and the presence of a non-ionic surfactant in the homogenization step.

*Lyons et al*. cannot cure the deficiencies of the combination of *Kumazawa* and *Ragland* as already discussed, because *Lyons et al*. does not teach or suggest any method for preparing cell wall fragments which comprises the features of the method of the present patent application.

*Lyons et al*. discloses the use of strain H37Rv to prepare cell wall fragments according to a substantially different method, which are used for other purposes than the treatment of tuberculosis in combination with other drugs.

*Kumazawa* addresses the preparation of a water soluble adjuvant from *M. tuberculosis* strain Aoyama B. The use of either this strain or H37Rv strain, as suggested by the Examiner in view of *Lyons et al.*, would not lead to the immunotherapeutic agent of the present patent application, which contains cell wall fragments insoluble in water and is substantially free of soluble cell compounds.

As claims 48, 49, 51, 52, 54 and 55 each depend, directly or indirectly, from claim 47 and thus incorporate all the limitations of the base claim, they, too, are patentably unobvious over the cited references for at least the same reasons as claim 47. Reconsideration and withdrawal of the rejection are respectfully requested.

II.4.- Rejection over *Kumazawa et al.* in view of *Ragland* and further in view of *Dhiman et al.*

Claim 56 stands rejected under 35 U.S.C. 103(a) as allegedly being obvious over *Kumazawa et al.* in view of *Ragland*, and further in view of *Dhiman et al.* (Indian J Exp Biol 1999; 37: 1157-66).

According to the Examiner, *Dhiman* supplements the combined teachings of *Kumazama* and *Ragland* by establishing it was well known in the art that liposome may be present in the mycobacteria cell wall composition as an adjuvant.

The Examiner concludes that it would have been obvious to an ordinarily skilled person in the art to include liposome in the composition of mycobacterial cell wall extracts with a reasonable expectation of success.

The Applicant respectfully disagrees.

*Dhiman* is a review article collecting different approaches for the control of tuberculosis disease, specially focused on defined proteins of bacterial origin, identified as targets for vaccine development.

Among the large quantity of data that is included in the article, *Dhiman* discloses that a cell wall composition in liposome shows a lower efficacy compared with the standard vaccine BCG (see page 1163, Table 4).

From that disclosure, the skilled person would not have been prompted to use cell wall fragments in liposome form because the efficacy is worse than the standard vaccination procedure.

Moreover, *Dhiman* cannot cure the deficiencies of the combination of *Kumazawa* and *Ragland* as already discussed, because *Dhiman* does not teach or suggest any method for preparing cell wall fragments which comprises the features of the method of the present patent application.

For these reasons, the Applicant respectfully requests the Examiner to withdraw the instant rejection.

II.5.- Rejection over *Kumazawa et al.* in view of *Ragland* and *Dhiman et al.* and further in view of *Parikh*

Claims 57-60 stand rejected under 35 U.S.C. 103(a) as allegedly being obvious over *Kumazawa et al.* in view of *Ragland*, and *Dhiman et al.*, and further in view of *Parikh* (US 5,785,975).

According to the Examiner, *Parikh* supplements the combined teaching of *Kumazawa*, *Ragland* and *Dhiman* by establishing it was known in the art that a liposome has many forms and components including sterols, phosphatidylcholine, and that a bacteria vaccine composition may comprise phosphatidylcholine liposome and vitamin E.

The Examiner concludes it would have been obvious to the skilled person in the art to include additional elements in the composition of the mycobacterial cell wall extracts with a reasonable expectation of success.

The Applicant respectfully disagrees.

*Parikh* describes phospholipid adjuvant compositions and vaccine formulations (Abstract), stating that examples of vehicles with adjuvant-like activities include water/oil emulsions, oil/water emulsions, microencapsulation, and liposomes (paragraph 15). In Example II, *Parikh* discloses a vaccine emulsion formulation comprising a mixture of beta-glucanphospholipid conjugate, phosphatidylcholine and vitamin E (paragraph 44).

However, *Parikh* does not cure the deficiencies of *Kumazawa* and *Ragland*, as already discussed, since *Parikh* neither teaches nor suggests any method for preparing cell wall fragments. Moreover, *Parikh* discloses a vaccine containing a beta-glucanphospholipid conjugate, which is not used in the invention of the present application.

The Examiner has relied on *Parikh* for the feature that the pharmaceutical composition in the form of liposomes further comprises vitamin E. Since, as discussed above, claims 47 and 54 are non-obvious over *Kumazawa* in view of *Ragland* and *Lyons* does not remedy the deficiencies of *Kumazawa* and *Ragland,* then dependent claims 57-60, which further limit the composition, must also be non-obvious.

For these reasons, the Applicant respectfully requests the Examiner to withdraw the instant rejection.

## III. Conclusion

Early and favorable action in the application is respectfully requested. If any issues should remain, the Examiner is encouraged to contact Applicants' legal representatives at the number listed below.

Respectfully submitted,

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